

## SPECIFICATION

Feed Additive for Domestic Animals

## TECHNICAL FIELD

The present invention relates to a feed additive for domestic animals, feed for domestic animals, and a method of raising domestic animals. More specifically, the present invention relates to a feed additive for domestic animals, feed for domestic animals, and a method of raising domestic animals, which reduce the number of deaths of domestic animals, increase the rate of raising and enable production of meat with high safety and good flavor.

## BACKGROUND ART

In recent years, as competition in producing domestic animals increases, economical and efficient production has been required. Given a current situation, it is hard to say that comfortable environments for domestic animals to be raised in are always provided. As a result, animals suffer from stress, so that they tend to develop diseases such as infectious diseases.

Conventionally, drugs such as antibiotics are used as measures against diseases and particularly against infectious diseases. However, such drugs are unfavorable because they reside in the body, regular addition of antibiotics invites the emergence of resistant microbes, or the like. Currently, such drugs tend not to be used when possible.

For pet animals (pets), various pet foods have been developed, but the purposes thereof are to mainly prevent obesity resulting from overeating, diabetes, and the like.

However, feed for domestic animals, which aims at production of meat or the like, is completely different from so-called pet foods, so that the following points must be considered.

There is a need to satisfy conditions such as:

- 1) since domestic animals are often raised in a group, feed for domestic animals must be acceptable for the entire group of the animals;
  - 2) feed for domestic animals must relieve stress in a group and prevent the spread of infectious diseases in a group, so as to improve the rate of raising;
  - 3) particularly in a state of raising a group of domestic animals, once resistant microbes emerge, diseases easily spread, and thus feed for domestic animals must not allow the emergence of resistant microbes;
  - 4) feed for domestic animals must make the flavor or the like of meat better rather than adversely affect the same, and be safe;
  - 5) in view of economic efficiency and the like, feed for domestic animals must exert its action in as small a quantity as possible; and
  - 6) a stable supply of feed for domestic animals must always be possible.
- Currently, feeds satisfying all of these conditions are extremely limited under the present situation.

## SUMMARY OF THE INVENTION

An object of the present invention is to provide a feed additive for domestic animals, feed for domestic animals, and a method for raising domestic animals, which relieve the stress of domestic animals and prevent the death thereof so as to increase the rate of raising and enable production of meat with high safety and good flavor.

As a result of intensive studies to achieve the above object, we have discovered that the "Maitake" mushroom (*Grifola*) satisfies the above conditions, and thus have completed the present invention.

That is, the present invention relates to:

- (1) a feed additive for domestic animals, comprising a *Grifola*-derived substance selected from one or more of dried *Grifola*, dried *Grifola* powder, and a *Grifola* extract mixed therein;
- (2) the feed additive for domestic animals of (1), wherein the dried *Grifola* is produced by heating and drying fresh *Grifola* by gradually increasing the temperature stepwise initially from approximately 60°C to finally

approximately 80°C;

(3) the feed additive for domestic animals of (1), wherein the Grifola extract is a Grifola extractive obtained by adding an alcohol to a water extract of fresh or dried Grifola or/and dried Grifola powder, leaving the resultant to stand, removing matter floating on or in the solution or adhering to the wall surface of a vessel, and concentrating the resultant;

(4) a feed additive for domestic animals, comprising a Grifola-derived substance selected from one or more of dried Grifola, dried Grifola powder, and a Grifola extract and a yeast-derived substance mixed therein;

(5) the feed additive for domestic animals of (4), wherein the yeast-derived substance is a dried yeast cell wall;

(6) the feed additive for domestic animals of (4), wherein and the Grifola-derived substance and the yeast-derived substance are mixed to result in a proportion of the former substance to the latter substance that is between 0.05 : 1.0 and 3.0 : 1.0;

(7) the feed additive for domestic animals of (6), wherein the yeast-derived substance is a dried yeast cell wall;

(8) the feed additive for domestic animals of any one of (1) to (7), wherein the domestic animals are chickens, swine, or cattle;

(9) a feed, comprising 0.05% by weight to 1% by weight of the feed additive for domestic animals of any one of (1) to (7) added thereto;

(10) a method for raising domestic animals, comprising providing 5 to 600 mg per day of the feed additive for domestic animals of any one of (1) to (7) per kg body weight of a domestic animal;

(11) the method for raising domestic animals of (10), wherein the domestic animals are chickens, swine, or cattle;

(12) a method for producing meat of domestic animals with good flavor, comprising providing the feed additive for domestic animals of any one of (1) to (7) to domestic animals;

(13) a method for producing meat of domestic animals with good flavor, comprising providing 5 to 600 mg per day of the feed additive for domestic animals of any one of (1) to (7) per kg body weight of a domestic animal; and

(14) the method for producing the meat of domestic animals of (12) or (13), wherein the domestic animals are chickens, swine, or cattle.

In addition, mixing of *Grifola* or a *Grifola* extract into pet foods for the purpose of improving and maintaining the glow of a pet hair coat or preventing lifestyle-related diseases such as obesity and diabetes is disclosed in JP Patent Publication (Kokai) No. 8-38069 A (1996) and JP Patent Publication (Kokai) No. 2001-69921 A (2001).

However, pets such as dogs and cats are placed in circumstances that can be said to be the intake of excess nutrition. Thus these inventions are significantly different from the present invention in terms of types of animals and purposes thereof, because the present invention aims at increasing the rate of raising chickens, swine, cattle, and other domestic animals for meat production. Furthermore, the feed additive and the feed of the present invention have a purpose differing from those of the above known examples in that they can have an effect not only on the improvement of animals' own health but also on the qualities of animal meat, including safety, flavor, and the like.

In the present invention, all types of *Grifola* including "Maitake" (*Grifola frondosa*), "Shiromaitake" (*Grifola albicans*), "Choreimaitake" (*Dendropolyporus umbellatus*), "Tonbimaitake" (*Grifola gigantea*), and the like can be used. In addition, both mycelia and fruit bodies of these types of *Grifola* can be used. Recently, artificial cultivation of the fruit bodies of "Maitake" (*Grifola frondosa*) has been made possible. It is preferable to use the fruit bodies of *Grifola* in terms of ensuring a stable supply of raw materials.

As dried *Grifola*, any *Grifola* prepared by sun drying, hot air drying, or freeze drying can be used. As a result of studies, it has been shown that dried products prepared by, for example, drying fresh *Grifola* by gradually increasing the temperature stepwise initially from approximately 60°C to finally approximately 80°C are preferable because of their good active oxygen-scavenging activity. Thus, the applicant has obtained a patent

(Japanese Patent No. 3260329).

As a Grifola-derived substance obtained as described above, dried Grifola, that is, Grifola in a dried form, can be used as it is, or can also be used in the form of small chips, small pieces, or fine pieces after appropriate grinding. However, it is more general to grind dried Grifola into powder using a milling apparatus or the like for use, because this leads to a broader application range. In addition, depending on the state of feed to which Grifola is added, Grifola powder can be appropriately selected from those with large particle sizes to Grifola finer powder with small particle sizes.

As a Grifola extract, extracts produced by standard methods using water, an alcohol, or the like can be used. The applicant has elucidated that a Grifola extractive obtained by adding an alcohol to the water extract of fresh or dried Grifola or dried Grifola powder, leaving the resultant to stand, removing matter floating on or in the solution or adhering to the wall surface of a vessel, and concentrating the resultant is preferable because of its good immunopotentiating action and active oxygen-scavenging activity (Japanese Patent Nos. 2859843 and 3260329).

The extraction method is performed at a temperature between room temperature and 135°C for 15 minutes to 3 hours. The short-time extraction is performed at 100°C or higher under pressure; for example, at about 120°C for about 30 minutes to about 1 hour under an atmospheric pressure between 1 and 2 using an autoclave. As water, any type of water such as distilled water, purified water, ion exchanged water, tap water, or natural water can be used. The amount of water used for the extraction is, for example, from about 4 volumes to about 20 volumes, based on 1 weight of dried Grifola or dried Grifola powder. When fresh Grifola is used, water is used in an amount from about 2 volumes to about 10 volumes, based on 1 weight of the same.

As an alcohol, methanol, ethanol, and the like can be used. An alcohol may be added to the extract in a final concentration between 20% and 70% by volume. An alcohol with water content between 0% and 50% can be

used. After the addition of the alcohol, the resultant solution is left to stand for 1 hour to 20 hours at a temperature between 1°C and 25°C, whereby matter floating on or in the solution or adhering to the wall surface of a vessel appears. The matter is then collected and removed from the solution by filtration, pipetting, scooping up with a meshed material, or similar methods.

The thus obtained extract can be used as dried extractive powder by conducting any conventional drying means such as concentration drying, spray drying, vacuum drying, or freeze drying. Furthermore, the extract can also be used after purification.

The dried Grifola, dried Grifola powder, and Grifola extract obtained as described above can be added independently to feed. To enable thorough mixing of the Grifola added to the feed, Grifola powder, Grifola extract or Grifola in the dosage form of powders mixed with an expander and a lubricant, granules, pellets, or the like prepared by standard methods can also be added to the feed.

When a case of using Grifola in the form of powder is explained as an example, an expander and a lubricant are added to the dried Grifola powder or the Grifola extract, and then the resultant is thoroughly mixed. At this time, if necessary, substances other than a Grifola-derived substance can be added.

As an expander such as lactose, starch, dextrin, or the like can be used. As a lubricant, light liquid paraffin or the like can be used. Particularly when the Grifola extract is used, it is preferable to mix the Grifola extract with an expander, because the Grifola extract becomes easily moistened.

A Grifola-derived substance contains  $\beta$ -glucan and has immunopotentiating action. As a result of searching for a substance having similar immunopotentiating action, we have discovered that it is more preferable to mix in a yeast-derived substance.

As a yeast-derived substance, those fermented by yeast (Saccharomyces cerevisiae) used in bread production, sake brewing, wine brewing or top fermentation beer brewing using a sugar-containing resource,

so-called beer yeast obtained upon bottom fermentation beer brewing using yeast, and the like can be used.

Furthermore, through the use of various types of yeast as raw materials, yeast-derived substances such as yeast extractives extracted by autolysis or the addition of enzymes, and furthermore, dried yeast cell walls (YCW marketed by Tanabe Seiyaku Co., Ltd.) obtained by separating yeast cell walls rich in polysaccharides such as glucan and mannan can be used.

Among yeast-derived substances, it is preferable to use dried yeast cell walls, particularly in terms of their good in vivo immunopotentiating action.

When a feed additive is produced by mixing a Grifola-derived substance with a yeast-derived substance, any compounding ratios can be selected. As a standard, it is better to select a compounding ratio of a Grifola-derived substance to a yeast-derived substance that is between 0.05 : 1.00 and 3.00 : 1.00. For example, when dried Grifola extract powder is used as a Grifola-derived substance, mixing can be performed based on a mixing ratio of the dried Grifola extract powder to a yeast-derived substance that is between about 0.05 : 1.00 and about 0.50 : 1.00. Alternatively, when dried Grifola or dried Grifola powder is used, mixing can be performed based on a mixing ratio of the dried Grifola or the dried Grifola powder to a yeast-derived substance broadly ranging from about 0.20 : 1.00 to about 3.00 : 1.00.

Feed additives obtained as described above are used by appropriately adding the additives to animal feed. The addition percentage is 0.05% by weight or more. The upper limit is the amount of the additive that an animal can ingest. In the case of domestic animals, economic efficiency should also be considered. Thus, it is considered reasonable that about 1% by weight be regarded as an upper limit.

For example, when 0.05% by weight to 1% by weight of the feed additive of the present invention is mixed into feed, standards regarding the amount of the additive to be provided per day to chickens, swine, cattle, or the like are as shown below.

Table 1

Standards regarding the amount of the additive to be provided

	Body weight	Feed (amount/day)	0.05% to 1% by weight of feed additive mixed in the feed (per animal)	0.05% to 1% by weight of feed additive mixed in the feed (per kg)
Chicken (broiler)	3 kg	175 g	87.5 to 1750 mg	29.2 to 583.3 mg
Swine	100 kg	3.5 kg	1.75 to 35 g	17.5 to 350.0 mg
Cattle (for fattening)	600 kg	7.5 kg	3.75 to 75 g	6.3 mg to 125.0 mg

As understood from Table 1, in the case of adding 0.05% by weight to 1% by weight of the feed additive to feed, the feed additive is provided in an amount within a range between approximately 5 mg and 600 mg per kg body weight, regardless of the type of domestic animal. This can be said to be a safe and appropriate amount.

"Domestic animals" used in the present invention include animals for use in production of meat in addition to chickens, swine, cattle, domestic ducks, and sheep. In particular, the present invention is effective for chickens, swine, and cattle.

#### BEST MODE FOR CARRYING OUT THE INVENTION

Next, examples will be given to specifically explain the present invention. In addition, the present invention is not limited by these examples.



[Example 1] Production of Grifola-derived substance

(1) Dried Grifola and dried Grifola powder

Artificially-cultivated fruit bodies of fresh Grifola frondosa were lined on the racks of a drying room with racks, and then dried by blowing hot air at approximately 60°C to approximately 80°C. By raising the temperature stepwise initially from 60°C to finally 80°C for almost 1 day so as to perform heating and drying, dried fruit bodies of Grifola frondosa were obtained. Subsequently, the dried Grifola frondosa was powdered with a milling apparatus.

(2) Grifola extract

10 kg of the dried fruit bodies of Grifola frondosa powder (hereinafter referred to as dried Grifola powder) was treated under pressure at 120°C with 100 L of purified water for 30 minutes, and then filtered, thereby obtaining 60 L of a blackish-brown extract. The solution was concentrated under reduced pressure to 25 L. When 28 L of 95% ethanol was added to the solution at room temperature, and then the resultant was left to stand for approximately 18 hours, brownish-red matter floating on or in the solution or adhering to the wall surface of the vessel was generated.

This matter was scooped up and removed with a metal mesh, thereby obtaining a brown solution. Alcohol was removed from the solution under reduced pressure, and then the resultant was concentrated under reduced pressure (24 to 42 kPa) at 70°C to 80°C until the Brix value of the solution became approximately 50%, thereby obtaining a heavy blackish-brown solution.

The solution was spray-dried using a rotating-disk-type spray dry system (centrifugal spray drying), thereby obtaining 1.6 kg of brown Grifola extractive powder peculiar to Grifola.

## [Example 2] Preparation of feed additive

## Feed additive 1

Dried Grifola powder	5 kg
<u>Lactose</u>	<u>5 kg</u>
Total	10 kg

## Feed additive 2

Dried Grifola powder	3 kg
Dried yeast cell wall (YCW)	4 kg
Lactic acid bacteria	0.05 kg
Light liquid paraffin	0.2 kg
<u>Lactose</u>	<u>remainder</u>
Total	10 kg

## Feed additive 3

Grifola extractive powder	0.2 kg
Dried yeast cell wall (YCW)	4.0 kg
Light liquid paraffin	0.2 kg
<u>Lactose</u>	<u>remainder</u>
Total	10 kg

Items in the above prescription were uniformly mixed well, so that each feed additive was prepared.

The additives prepared as described above can be directly mixed into feed and then used. If necessary, the additives can be prepared in the form of granules, soft pellets, dry pellets, or the like by standard methods, and then used.

## [Example 3] Raising test results and flavor and organoleptic test for produced meat

## (1) Raising test results for chickens

0.2% by weight of the feed additive 1 was added to the feed for test

group 1, and 0.2% by weight of the feed additive 2 was added to the feed for test group 2. A group provided with feed to which no feed additive had been added was used as a control group. The test was conducted from September to October. As a result, results as shown in the following Table 2 were obtained.

Table 2  
Raising test results for chickens

	Test Group 1	Test Group 2	Control Group
Number of incoming chicks	12750	12750	12450
Number of chickens shipped	12107	12359	11227
Rate of raising	94.96	96.93	90.18
Average body weight (kg)	2.919	2.934	2.911
Age in day	53.35	52.69	53.06
Feed-requiring rate	2.085	2.054	2.206
PS*	249.2	262.8	224.3
Waste ratio	1.51	1.44	1.80

$$*PS = \frac{\text{Rate of raising} \times \text{Average body weight}}{\text{Age in day} \times \text{Feed-requiring rate}} \times 100$$

As is also clear from the above results, test groups 1 and 2 showed excellent results in terms of rate of raising, PS value, waste ratio, and the like compared with those of the control group. This was the result of a low number of deaths due to stresses such as infectious diseases.

## (2) Flavor test results

Five chickens were randomly sampled from the chickens shipped of test group 2 and the control group, respectively, and then sacrificed. For odor and taste (the smell peculiar to chicken meat) of the breast meat, a total of 20 subjects consisting of 14 male subjects and 6 female subjects, whose age  ~~ranged from 30 to 70 years old, were subjected to a simple 3-stage~~ organoleptic test involving comparison of the test group with the control group. Thus, the following results were obtained.

## I. Raw meat            Odor (Comparison of the smell peculiar to chicken)

- (1) Smelled unsavory compared with the control: 1 out of 20 subjects
- (2) Unable to say which was which: 7 out of 20 subjects
- (3) Improved compared with the control: 12 out of 20 subjects

## II. Directly boiled meat without seasoning

## Odor (Comparison of the smell peculiar to chicken)

- (1) Smelled unsavory compared with the control: 1 out of 20 subjects
- (2) Unable to say which was which: 5 out of 20 subjects
- (3) Improved compared with the control: 14 out of 20 subjects

## Taste (Comparison of the taste peculiar to chicken)

- (1) Tasted unsavory compared with the control: 1 out of 20 subjects
- (2) Unable to say which was which: 4 out of 20 subjects
- (3) Improved compared with the control: 15 out of 20 subjects

## III. Directly grilled meat without seasoning

## Odor (Comparison of the smell peculiar to chicken)

- (1) Smelled unsavory compared with the control: 0 out of 20 subjects
- (2) Unable to say which was which: 2 out of 20 subjects
- (3) Improved compared with the control: 18 out of 20 subjects

## Taste (Comparison of the taste peculiar to chicken)

- (1) Tasted unsavory compared with the control: 0 out of 20 subjects
- (2) Unable to say which was which: 3 out of 20 subjects

(3) Improved and tasted good compared with the control: 17 out of 20 subjects

As shown above, it was revealed that the test group was improved in terms of odor and taste compared with those of the control group.

### (3) Results of the effect of body weight gain in weaner piglets

Weaner piglets were observed, concerning the presence or the absence of an effect of body weight gain, wherein a group to which feed comprising 0.2% by weight of feed additive 2 mixed therein had been provided and a control group to which only feed had been provided were compared.

(Test groups)

Test group: 55 female piglets      Feed comprising 0.2% by weight of feed additive 2 mixed therein

Control group: 27 female piglets      Only feed

(Test period)

27- (at the time of weaning) to 90-day-old piglets

(Feed during the test period)

I. 27 days to 60 days after birth      Synthetic milk A

II. 61 days to 70 days after birth      Synthetic milk B

III. 71 days to 90 days after birth      Feed for piglets

Table 3

Changes in average body weight

	At start time of test	At end time of test
Test group	7.92 kg	44.2 ± 5.1 kg
Control group	7.92 kg	41.4 ± 6.8 kg

From the above results, clear tendencies of body weight gain were observed in the test group.

Industrial Applicability

The feed additive for domestic animals, the feed for domestic animals, or the method for raising domestic animals of the present invention is safe for domestic animals, enhances the immune strength of domestic animals, reduces the number of deaths of domestic animals due to stress, infectious diseases, or the like so as to increase the rate of raising, and has an effect of making the flavor of the meat of domestic animals better.

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